

so that a population of cells comprising avian EG cells is obtained.

14. (Four Times Amended) A method of producing chimeric avians comprising:

- (i) isolating primordial germ cells (PGCs) from an avian;
- (ii) culturing the PGCs in the absence of a feeder layer in a tissue culture medium containing at least the following growth factors:
  - (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF)for a sufficient time to produce embryonic germ (EG) cells;
- (iii) transferring cells produced by step (ii) comprising said EG cells into a recipient avian embryo; and
- (iv) obtaining a germline and somatic cell chimeric avian.

25. (Thrice Amended) A method of producing germline chimeric avians comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) maintaining said PGCs for a period of at least fourteen days in a tissue culture medium containing at least the following growth factors:
  - (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF);
- (iii) transferring PGCs produced by step (ii) into a recipient avian embryo; and

(iv) obtaining germline chimeric avians.

26. (Four times Amended) A method of producing germline and somatic cell chimeric avians which comprises:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) maintaining said PGCs in a tissue culture medium containing at least the following growth factors:
  - (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF),for a sufficient time to produce embryonic germ (EG) cells;
- (iii) transferring cells produced by step (ii) comprising said EG cells into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs;
- (iv) allowing said recipient avian embryo containing said transferred EG cells to develop into a germline and somatic cell chimeric avian.

27. (Thrice Amended) A method for producing avian embryonic germ (EG) cells comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
  - (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),

- (3) stem cell factor (SCF) and
- (4) insulin-like growth factor (IGF)

so that a population of cells comprising avian EG cells is produced.

28. (Thrice Amended) A method for producing a germline chimeric avian comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
  - (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),
  - (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF);
- (iii) transferring said PGCs produced by step (ii) into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs;
- (iv) allowing said recipient avian embryo containing said transferred PGCs to develop into a germline chimeric avian.

29. (Thrice Amended) A method for producing a germline chimeric avian comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
- (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
  - (1) leukemia inhibitory factor (LIF),
  - (2) basic fibroblast growth factor (bFGF),

- (3) stem cell factor (SCF) and
  - (4) insulin-like growth factor (IGF);
- (iii) transferring said PGCs produced by step (ii) into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs; and
- (iv) allowing said recipient avian embryo containing said transferred PGCs to develop into a germline chimeric avian.

30. (Thrice Amended) A method for producing germline or somatic cell chimeric avians comprising:

- (i) isolating primordial germ cells (PGCs) from a Stage XII-XIV avian embryo;
  - (ii) culturing said PGCs for a period of at least fourteen days in tissue culture in the absence of a feeder layer in a culture medium comprising:
    - (1) leukemia inhibitory factor (LIF),
    - (2) basic fibroblast growth factor (bFGF),
    - (3) stem cell factor (SCF) and
    - (4) insulin-like growth factor (IGF),for a sufficient time to produce embryonic germ (EG) cells;
- (iii) transferring said cells produced by step (ii) comprising EG cells into a recipient avian embryo of the same species as the avian used to obtain said isolated PGCs; and
- (iv) allowing said recipient avian embryo containing said transferred EG cells to develop into a germline or somatic cell chimeric avian.